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Hormones and Prostate Cancer: What's Next?

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INTRODUCTION

Although there is abundant biologic evidence implicating a pivotal role of androgens in the development of prostate cancer, epidemiologic data to date are inconclusive (1–3). Methodological limitations, including intra-person and laboratory variation and timing of measurement, could, in part, contribute to the mixed results. To provide insights and directions for future studies, this presentation briefly reviews current perspectives on hormones and prostate cancer, discusses reasons for inconsistent results in epidemiologic studies, summarizes methodological issues related to the validity of serum-based studies, and provides specific suggestions for future studies. Since there are several meta-analyses and reviews on hormones and prostate cancer (2, 4–7), this review will focus primarily on conceptual and methodological issues. In addition to androgens, the role of several other hormones, including estrogen, insulin, and leptin, and sex hormone-binding globulin will be discussed briefly. Insulin-like growth factors and vitamin D are not discussed here since they are reviewed in two separate presentations in this special issue of *Epidemiologic Reviews*; these presentations are titled "Insulin-like Growth Factor and Prostate Cancer" and "Dairy Products, Calcium, and Vitamin D and Risk of Prostate Cancer." Studies of genetic polymorphisms involved in androgen biosynthesis, metabolism, and transport are not reviewed, but some of the genetic markers are mentioned briefly in the context of future studies. A more in-depth review of these hormone-related polymorphisms is provided in the presentation titled "Molecular Epidemiology of Hormone-metabolic Loci in Prostate Cancer" in this issue of *Epidemiologic Reviews*. In addition, a comprehensive review of hormones and hormone-related genes is presented elsewhere (3).

BACKGROUND

Biosynthesis and metabolism of androgens

Androgens, male sex hormones, are formed in the testes and adrenal glands, as well as in peripheral tissues such as the prostate and skin. The biosynthesis and metabolism of androgens are shown in figure 1. Also shown are the enzymes involved in the metabolic pathways and the genes encoding these enzymes. Testosterone and dihydrotestosterone are the two most important androgens of adult males. Testosterone is the major male androgen in circulation, while dihydrotestosterone is the principal androgen in tissue. In healthy adult men, 90 percent of the circulating levels of testosterone is secreted by the Leydig cells of the testes and 5–10 percent from the adrenal glands. In the circulation, about 44 percent of testosterone is bound firmly to sex hormone-binding globulin, 54 percent is bound loosely to albumin, and only 1–2 percent is in a free state. Unlike testosterone, only 25 percent of dihydrotestosterone in the circulation is secreted by the testes; most dihydrotestosterone (65–75 percent) arises from conversion of testosterone in peripheral tissue (such as prostate and skin) through the action of the enzyme 5α-reductase. In humans, there are two 5α-reductase isoenzymes. The type 1 enzyme (encoded by the *SRD5A1* gene) is expressed mostly in skin and hair, while the type 2 enzyme (encoded by the *SRD5A2* gene) is located primarily in androgen target tissue, including genital skin and prostate (8).

Androgen metabolism within the prostate

In men, the prostate is a major site of non-testicular production of dihydrotestosterone, which is derived primarily from testosterone. Figure 2 shows the metabolism of androgens within the prostate. Within the prostate, testosterone is converted irreversibly to dihydrotestosterone by 5α-reductase type 2. In addition to reduction of testosterone, dihydrotestosterone can be formed from androstenedione by a two-step reduction in which 5α-reductase type 2 converts androstenedione to 5α-androstane-3,17-dione, which is then converted to dihydrotestosterone by 17β-hydroxysteroid dehydrogenase type 3 (encoded by the *HSD17B3* gene) in a reversible reaction (9). Within the prostate, dihydrotestosterone can undergo further reversible reduction to form either 5α-androstane-3α,17β-diol (3α-diol) by the enzyme 3α-hydroxysteroid dehydrogenase (encoded by the *HSD3A* gene), or 5α-androstane-3β,17β-diol (3β-diol) by the type 2 enzyme 3β-hydroxysteroid dehydrogenase (encoded by the *HSD3B2* gene). 3α-diol, also a potent androgen, can be conjugated through the action of glucuronide transferase in an irreversible reaction yielding 5α-androstane-3α, 17β-diol, glucuronide (3α-diol G), a terminal metabolite of dihydrotestosterone. Inactivation of dihydrotestosterone in the prostate by reduction to either

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Abbreviations: 3α-diol: 5α-androstane-3α,17β-diol; 3α-diol G, 5α-androstane-3α, 17β-diol: glucuronide; 3β-diol: 5α-androstane-3β, 17β-diol.

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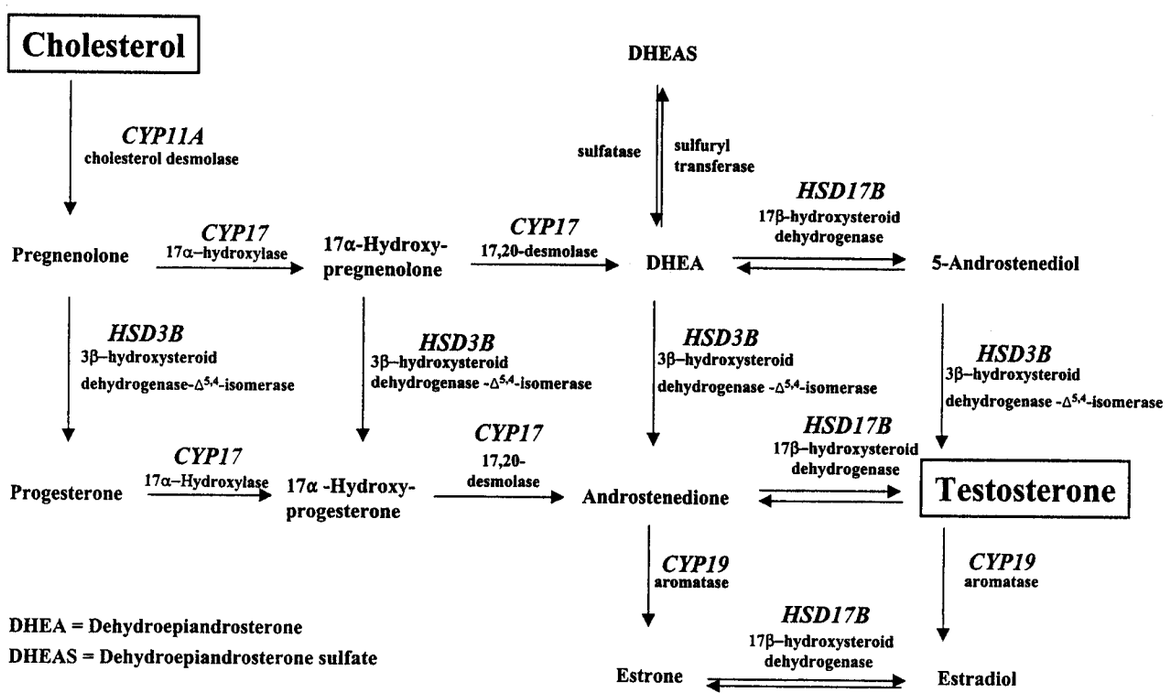

FIGURE 1. Androgen pathways: biosynthesis and metabolism. Abbreviations: DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate.

3α-diol or 3β-diol is an important determinant of intracellular dihydrotestosterone concentration and a potential modulator of androgenic activity in the prostate.

Dihydrotestosterone levels in tissue are several times higher than the levels of testosterone, but serum levels of dihydrotestosterone are only 10 percent of the serum levels of testosterone because most dihydrotestosterone is produced in tissue. In epidemiologic studies, serum levels of 3α-diol G are commonly used as an indirect measure of 5α-reductase enzymatic activity, since it is not feasible to measure tissue levels of testosterone and dihydrotestosterone in cases and healthy subjects. The current belief is that serum levels of 3α-diol G reflect enzyme activities of both steroid 5α-reductase types 1 and 2. However, in studies of men treated with finasteride, a 5α-reductase type 2 inhibitor, serum levels of 3α-diol G decrease concomitantly with finasteride treatment, suggesting that 3α-diol G levels predominantly reflect the activity of the type 2 enzyme (10).

Intraprostatic androgenic action

The function of dihydrotestosterone in the prostate is mediated through the androgen receptor protein. Within the prostate, dihydrotestosterone binds to androgen receptor to form an intracellular dihydrotestosterone-androgen receptor complex (testosterone binds to androgen receptor with a much lower affinity). The dihydrotestosterone-androgen receptor complex then binds to prostate DNA, where it activates transcription of several genes with androgen-response elements in their promoters, thus inducing DNA synthesis and cellular proliferation (3) (figure 3). It should be noted that androgenic action within the prostate is determined by tissue dihydrotestosterone concentration as well as several other factors, including the amount of testosterone, the activity of several key enzymes, levels of the androgen receptor protein and its coactivators, levels of growth factors and their receptors, and perhaps other factors yet to be identified (figure 3).

The role of androgen receptor both in normal prostate growth and in prostate cancer is well recognized. Experimental studies have shown that in normal cells, the length of the CAG trinucleotide repeat length in the *AR* gene is related to transactivation of androgen receptor (11), thereby affecting androgenic action and the growth of prostate cells. Currently it is not known how much dihydrotestosterone is necessary to saturate the androgen receptor protein within the prostate and trigger the androgen signaling pathway to induce cellular proliferation. However, it has been suggested that the androgen receptor protein may play a more critical role in androgenic action than the dihydrotestosterone ligand, since among prostate cancer patients undergoing androgen ablation treatment, a minute amount of adrenal androgen can trigger a cascade of androgenic action through the increased hypersensitivity of mutant androgen receptors (12, 13). It has also been shown that in cancer cells in the absence of androgen, non-androgen hormones (including estrogens and insulin-like growth factors) in combination with androgen receptor can trigger andro-

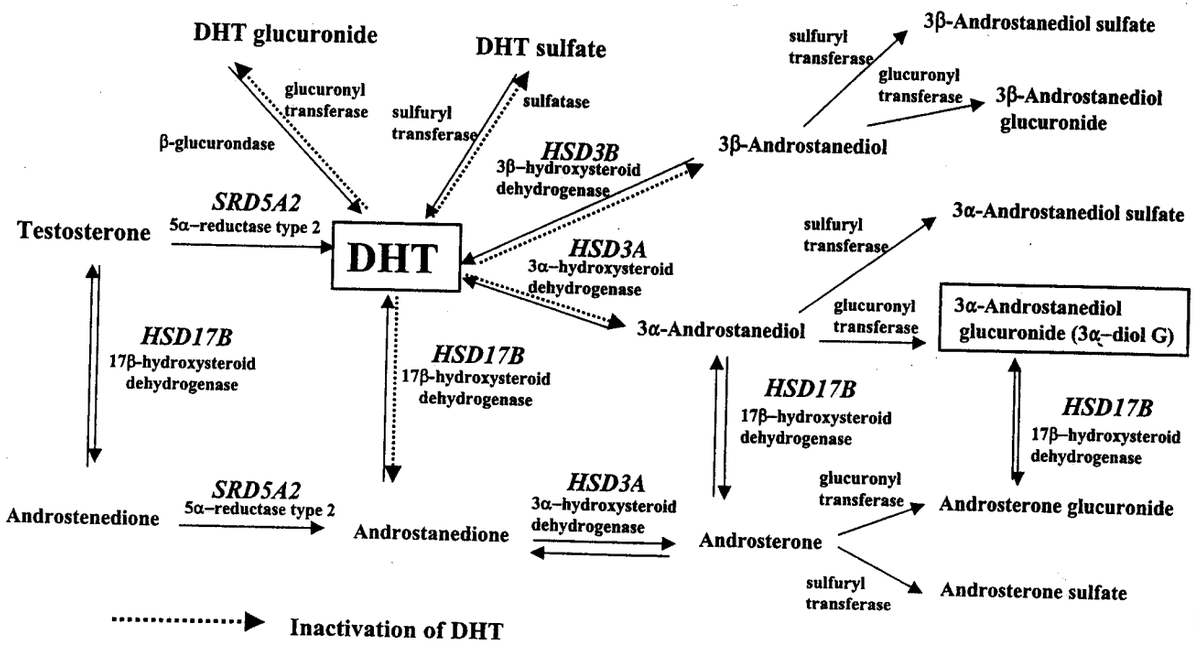

FIGURE 2. Androgen metabolism within the prostate. Abbreviation: DHT, dihydrotestosterone.

genic action (14, 15). Together, these data support the concept that ligands other than androgens can activate androgen receptor or, in some instances, receptor activation may be ligand-independent (12). In both situations, receptor activation can lead to promotion of tumor growth independent of androgens. It is important to point out that although the development of androgen-independent growth is a common and important clinical event in advanced prostate cancer (13), it is not clear whether such androgen-independent growth also reflects mechanisms that operate during earlier and more etiologically relevant stages of prostate carcinogenesis. If saturation of androgen receptor is a more critical step in cellular proliferation than the levels of dihydrotestosterone, a threshold effect of dihydrotestosterone would exist, and, in epidemiologic studies, it would be difficult to demonstrate a clear dose-response relation between levels of dihydrotestosterone, either in serum or in tissue, and prostate cancer.

In addition to androgen receptor, androgen receptor-associated proteins (androgen receptor coactivators) can interact with androgen receptor protein to increase androgenic action substantially within the prostate. In vitro studies have shown that certain androgen receptor coactivators, such as ARA 70 or ARA 55, p160, BRCA1, AIB1, and CBP, can enhance androgen receptor transcriptional activity by five- to 10-fold (16, 17). Currently, the amount of androgen receptor coactivators can be measured only through immunostaining in prostate tissue, making it difficult to assess androgen receptor and androgen receptor coactivator levels in epidemiologic studies. Two studies have investigated the polymorphisms of AIB1 in prostate cancer (18; A. W.Hsing,, Y. T. Gao, G. Wu, et al., unpublished data), but no epidemiologic studies have directly investigated the role of androgen receptor coactivators.

EPIDEMIOLOGIC EVIDENCE

Serum-based studies

Although androgens have been a central hypothesis in prostate cancer etiology for decades, data from epidemiologic studies have been mixed. To date, twelve prospective studies have investigated the role of serum androgens (19–30) (table 1), but only one (25) was able to show definitively that men with higher serum levels of testosterone have a higher risk of prostate cancer. Testosterone and dihydrotestosterone are measured in almost all studies. Two studies found a suggestive (nonsignificant) association of the ratio of testosterone to dihydrotestosterone, an indirect measure of steroid 5α-reductase 2 activity, thus suggesting a role for the 5α-reductase 2 enzyme (19, 21). 3α-diol G is measured in more recent studies as a surrogate marker for androgen and steroid 5α-reductase 2 activity within the prostate (24–30). Thus far, there are no consistent data to support the role of 3-α diol G.

The difficulties in demonstrating positive associations between serum levels of androgens and prostate cancer in epidemiologic settings can partly be explained by several methodological limitations (table 2), including limited statistical power in most studies, the relatively small number of incident cases in follow-up studies (<150 cases), the relatively small differences (10–15 percent) in mean serum lev-

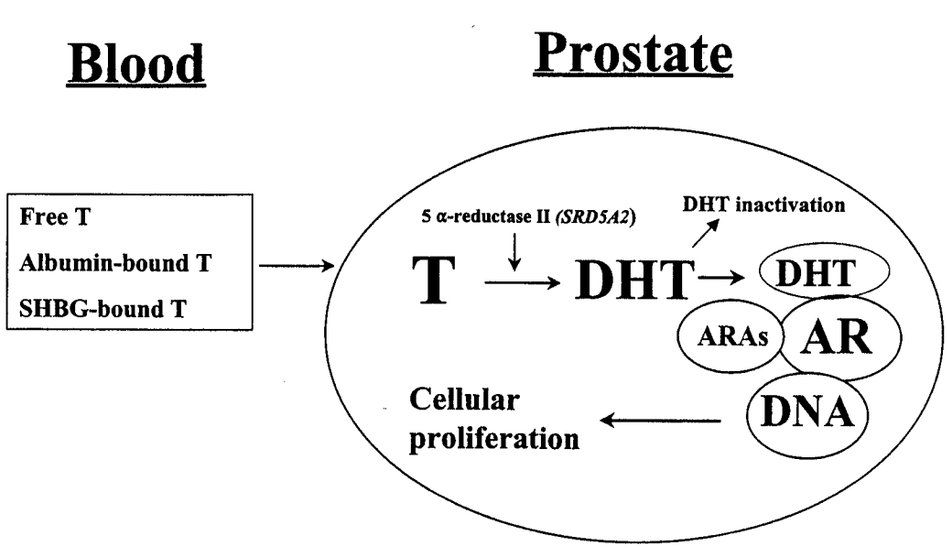

FIGURE 3. Androgenic action within the prostate. Abbreviations: AR, androgen receptor; ARA, androgen receptor-associated proteins; DHT, dihydrotestosterone; SHBG, sex hormone-binding globulin; T, testosterone.

els of hormones between cases and controls, and the somewhat large (5–15 percent) laboratory variations in intra- and inter-assays of serum hormone (31).

In addition, few studies have controlled adequately for confounding. For example, in the study of Gann et al. (25), the largest study to date and the only study that showed a clear positive relation between serum testosterone and prostate cancer risk, the positive association with serum testosterone and the inverse association with estradiol were not evident until after the effect of sex hormone-binding globulin was adjusted for. Due to the high correlation between testosterone and sex hormone-binding globulin and the potential of collinearity, it is not clear whether mutual adjustment of serum hormones and carrier proteins is the best approach statistically to assess an independent effect of a specific hormone. However, the example of Gann et al. (25) clearly illustrates the importance of measuring several hormones simultaneously in order to examine the individual and combined effects of these hormones. Similarly, several other factors, including body size, physical activity, diabetes, and benign prostatic hyperplasia, that might impact serum levels of hormones and have been linked to prostate cancer have not been adjusted for in previous nested case-control studies. Some of these factors, such as central obesity, may be intermediate variables and involved in the causal pathway. Thus, mutual adjustment may not be the best approach. Perhaps the effect of hormones in obese and non-obese subjects should be evaluated separately, since obesity and central obesity have a substantial impact on hormone metabolism. Confounding and interaction are issues that have not been addressed fully in most previous studies. In future studies, the interrelations among serum hormones and several other factors should be evaluated prior to establishing a model to assess the independent or joint effect of serum androgens and related factors.

When several hormones involved in the metabolic pathways are measured simultaneously in the same study, in addition to evaluating the effect of a specific hormone, the potential role of hormone imbalance can be investigated. Serum levels of testosterone, free testosterone, and dihydrotestosterone decrease, while serum levels of estradiol and sex hormone-binding globulin increase, with advancing age (32–34) (figure 4). These progressive changes coincide with the rise of prostate cancer incidence starting at approximately 50 years of age, suggesting that factors other than androgens or the imbalance of androgen/estrogen may be involved in the progression of latent prostate tumors to clinically overt cancer.

Although nested case-control studies are generally considered to be the best study design, several methodological issues deserve some attention. First, the potential disease effect on serum levels of hormones, though minimized, is not completely ruled out in some studies, particularly in those studies with a shorter follow-up, since currently it is not known at which point during the natural history of prostate cancer malignant tumors begin to impact on androgen biosynthesis and metabolism. Most prostate cancers are slow-growing with a long latency; the onset of cancer (or the presence of malignancy) probably occurs long before the tumor is diagnosed. Although the standard practice is to exclude cancer cases diagnosed within the first few years of blood collection from data analysis, to minimize the potential disease effect, the optimal cutoff is not known. Currently, there are no data comparing pre- and postdiagnostic serum levels of androgens within the same subject to provide some insight into the extent of changes in hormone

TABLE 1. Summary of serum hormones and prostate cancer

Study type	Study (reference no.) and year	Population	Sample size	Hormones	Results*	
					OR* or RR*	95% CI*
			Androgens			
Prospective cohort	Barrett-Connor et al. (20), 1990	US men	57 cases; 951 controls	Testosterone	RR = 1.00†	0.70, 1.43
				Androstenedione	RR = 1.26†	1.04, 1.54
	Mohr et al., (30), 2001	US men	70 cases; 1,506 controls	Testosterone	490 vs. 510 ng/dl (NS*)‡	
				Free testosterone	10 vs. 9 ng/dl (NS)‡	
				Dihydrotestosterone	25 vs. 23 ng/dl (NS)‡	
				3α-androstanediol glucuronide	660 vs. 710 ng/dl (NS)‡	
Nested case-control	Nomura et al. (19), 1988	US Japanese men	98 cases; 98 controls	Testosterone	OR = 0.99 (NS)§	
				Dihydrotestosterone	OR = 0.66 (NS)§	
	Hsing and Comstock (21), 1993	US white men	98 cases; 98 controls	Testosterone	449.7 vs. 440.8 mg/dl (NS)‡	
				Dihydrotestosterone	52.6 vs. 52.8 ng/dl (NS)‡	
	Comstock et al. (22), 1993	US white men	81 cases; 81 controls	Dehydroepiandrosterone	OR - 0.94 (NS)¶	
	Carter et al. (23), 1993	US men	16 cases; 16 controls	Testosterone	NS#	
	Nomura et al. (24), 1996	US Japanese men	141 cases; 141 controls	Testosterone	OR = 1.03¶	0.51, 2.07
				Non-sex hormone-binding globulin-bound testosterone	OR = 1.09¶	0.48, 2.51
				Dihydrotestosterone	OR = 0.82¶	0.41, 1.65
				3α-androstanediol glucuronide	OR = 1.37¶	0.73, 2.55
	Gann et al. (25), 1996	US white men	222 cases; 390 controls	Androstenedione	OR = 1.24¶	0.62, 2.47
				Testosterone	OR = 2.60¶,**	1.34, 5.02
				Dihydrotestosterone	OR = 0.71¶,**	0.34, 1.48
				3α-androstanediol glucuronide	OR = 1.60¶,**	0.93, 2.76
	Guess et al. (26), 1997	US men	106 cases; 106 controls	Testosterone	OR = 1.00††	0.75, 1.34
				Non-sex hormone-binding globulin-bound testosterone	OR = 1.14††	0.86, 1.50
				3α-androstanediol glucuronide	OR = 1.16††	0.86, 1.56
	Vatten et al. (27), 1997	Norwegian men	59 cases; 180 controls	Testosterone	OR = 0.83¶,‡‡	0.36, 1.93
				Dihydrotestosterone	OR = 0.83¶,‡‡	0.36, 1.94
				3α-androstanediol glucuronide	OR = 1.10¶,‡‡	0.41, 2.90

	Dorgan et al. (28), 1998	Finnish men	116 cases; 231 controls	Testosterone	OR = 0.8¶	0.4, 1.5
				Non-sex hormone-binding globulin-bound testosterone	OR = 1.1¶	0.6, 2.1
				Dihydrotestosterone	OR - 0.7¶	0.4, 1.3
				3α-androstanediol glucuronide	OR = 1.2¶	0.6, 2.3
				Androstenedione	OR = 1.0¶	0.5, 1.9
				Dehydroepiandrosterone	OR = 1.2¶	0.6, 2.3
	Heikkila et al. (29), 1999	Finnish men	166 cases; 300 controls	Testosterone	OR = 1.23¶	0.55, 2.76
				Androstenedione	OR = 0.92¶	0.49, 1.72
			Non-androgenic hormones			
Prospective cohort	Barrett-Connor et al. (20), 1990	US men	57 cases; 951 controls	Estrone	RR = 1.09†	0.86, 1.39
				Sex hormone-binding globulin	RR = 1.04†	0.80, 1.34
Nested case-control	Nomura et al. (19), 1988	US Japanese men	98 cases; 98 controls	Estrone	OR = 0.89 (NS)§	
				Sex hormone-binding globulin	OR = 0.85 (NS)§	
	Hsing and Comstock (21), 1993	US white men	98 cases; 98 controls	Estrone	35.2 vs. 35.5 pg/ml (NS)‡	
	Dorgan et al. (28), 1998	Finnish men	116 cases; 231 controls	Estrone	OR = 0.8¶	0.4, 1.5
				Sex hormone-binding globulin	OR = 0.8¶	0.5, 1.5
	Gann et al. (25), 1996	US white men	222 cases; 390 controls	Sex hormone-binding globulin	OR = 0.46¶	0.24, 0.89
	Carter et al. (23), 1995	US men	16 cases; 16 controls	Sex hormone-binding globulin	NS#	
Case-control	Lagiou et al. (58), 1998	Greek men	43 cases; 48 controls	Leptin	OR = 1.6†, §§	0.8, 3.2
	Stattin et al. (56), 2001	Swedish men	149 cases; 298 controls	Insulin	6.1 vs. 6.0 mIU/ml (NS)‡	
				Leptin	OR = 0.70¶¶	0.32, 1.55
	Hsing et al. (57), 2001	Chinese men	128 cases; 328 controls	Insulin	OR = 2.81§, ##	1.52, 5.17
				Leptin	OR = 1.10§, ##	0.59, 2.07

* Abbreviations: RR, relative risk; OR, odds ratio; CI, confidence interval; NS, not significant.
† Risk estimate for one standard deviation increase.
‡ Means or medians comparing cases vs. controls.
§ Odds ratio comparing highest to lowest tertiles.
¶ Odds ratio comparing highest to lowest quartiles.
# Means not significantly different between cases and controls at each of three levels of years before diagnosis.
** Mutually adjusted for testosterone, 3α-androstanediol glucoronide, estradiol, and sex hormone-binding globulin.
†† OR per 1 quartile increase
‡‡ Adjusted for age
§§ Adjusted for age, education, anthropometric factors, sex hormones, and insulin-like growth factor I.
¶¶ Odds ratio comparing highest to lowest quintiles.
Adjusted for age, education, and anthropometric factors.

TABLE 2. Factors related to the inability to detect an association of serum androgens and prostate cancer in epidemiologic studies

Factors	Comments
	Prospective studies (nested case-control studies)
1. Exposure assessment	
Validity and reproducibility of assays	Most studies used radioimmunoassays to measure hormones. Many have intra- and inter-assay variations larger than 10%
Collection of blood samples	Some studies used non-fasting blood samples and did not control for differences in hormone levels related to diurnal variation (intra- and inter-person variation)
Etiological relevant time periods	The etiologically relevant time for hormone levels in relation to prostate cancer is not clear. Most prospective studies collect baseline blood samples in men during their 40s or 50s. If this is not an etiologically relevant time period, the measurement may not represent critical exposure
Hormone profile	Most studies measured two or three key hormones. However, since many of these hormones are interrelated, it may be necessary to measure several hormones at the same time to assess an independent effect of a particular hormone
II. Outcome	
Case definition	Cases are heterogeneous in terms of clinical stage and histologic grade of tumors Earlier studies (pre-prostate-specific antigen era) included mostly advanced cases, while studies after 1986 included a large number of screen-identified localized and low-grade cases. The hormone associations may be different for different types of cases Comparison of results between studies is sometimes difficult
III. Statistical issues	
Sample size	Sample size is generally too small (usually less than 150) to examine a relatively small (<10%) case-control difference and to investigate the joint effects of several hormones at the same time
Data analysis	Some studies excluded controls with elevated levels of prostate-specific antigen, while some did not have any information on the number of controls who might have had latent prostate tumors Some studies measured several hormones simultaneously and controlled for the effect of sex hormone-binding globulin or other hormones Currently, it is not clear what hormones or binding proteins should be adjusted for in order to estimate the net effect of androgen Most studies did not control for the presence of benign prostatic hyperplasia, diabetes, or other factors that might affect circulating levels of hormones It may be necessary to stratify the analysis by the obesity index (body mass index). The hormone association among obese and lean subjects may be different
	Case-control studies
Methodological issues	Many are similar to those listed for prospective studies The inherent limitation of cross-sectional design is the potential of disease to affect the measurement of hormones (exposure), although the common belief that there is a difference between pre- and postdiagnostic levels of hormones has not been validated It is difficult to establish a temporal relation between serum hormones and prostate cancer in case-control studies

levels in relation to malignant growth. And although such a comparison would be informative, there are numerous methodological issues, such as treatment effects, stress related to surgery, and weight loss related to malignancy, that may affect serum levels of hormones in postdiagnostic blood, thereby influencing the validity of the comparison between pre- and postdiagnostic serum androgens.

Second, it is not known whether an etiologically relevant time period of exposure can be captured in nested case-control studies, since such a time period in prostate carcinogenesis has not been identified. Most prospective studies collect baseline blood samples from men in their 40s and 50s and follow them for 10 to 20 years for cancer outcome (figure 4). Hormone measurements in these studies represent hormonal status (exposure) between the 5th and 6th decades of life. If hormone levels during these 20 years are etiologically relevant, they are probably associated with progression of latent tumors to clinically significant prostate cancer, because many men in this age group may already have histologic evidence of latent tumors. In addition, prostatic intraepithelial neoplasia, a putative precursor lesion, can be identified in men as early as in their late 30s (35). Because at three points during a man's lifetime (the prenatal period, puberty, and at approximately 50 years of age) the prostate epithelial cells undergo substantial proliferation and differentiation (36), it has been suggested that hormonal levels during these critical

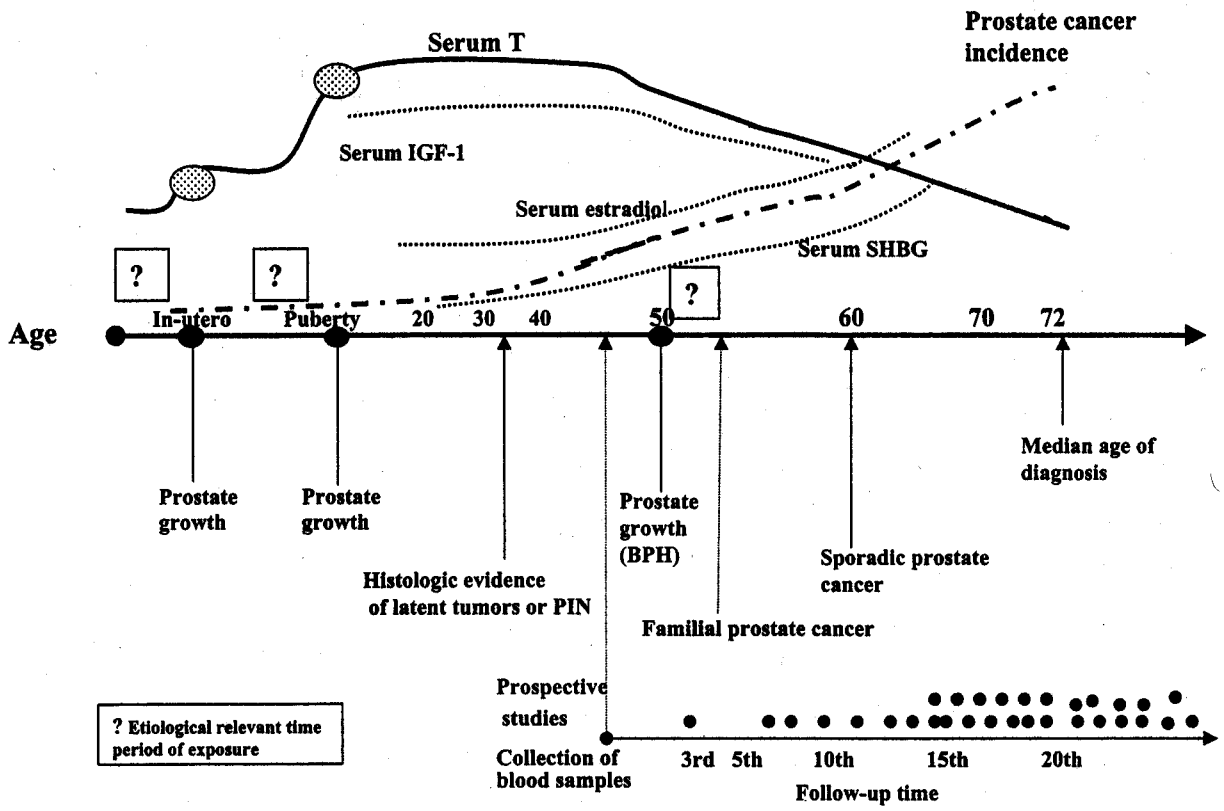

FIGURE 4. Conceptual diagram of serum levels of hormones, age-related prostate growth, and prospective studies with collection of baseline biologic samples. Abbreviations: BPH, benign prostatic hyperplasia; IGF-1, insulin-like growth factor-1; PIN, prostatic intraepithelial neoplasia; SHBG, sex hormone-binding globulin; T, testosterone.

time periods may be etiologically more relevant (37). Thus, if exposure in early life during the prenatal or peri-puberty periods is most critical, most nested case-control studies would miss these etiologically relevant periods of exposure.

Third, it is not known whether one single measure of hormones in the 5th and 6th decades of life is indicative of one's usual exposure during adult life. It is possible that a single measure of hormones may reflect one's hormonal status over time during adult life and accurately classify a subject into a specific exposure category. However, if there are substantial changes in lifestyle or body size over time, which can alter hormone metabolism, one single measure of hormones may not be sufficient to capture one's hormone profile or changes over time. In addition, this change in hormonal status over time is likely to occur more frequently in cases than in controls, since several lifestyles that influence hormonal metabolism (e.g., obesity and physical inactivity) have also been linked to prostate cancer. Furthermore, even without changes in lifestyle, after age 30 a man's serum levels of testosterone decrease and his serum levels of estradiol and sex hormone-binding globulin increase with advancing age (32–34). These age-dependent progressive changes in serum hormones result from both physiologic (such as decline in testicular and pituitary functions) and physical (such as increase in obesity, diabetes, and loss of muscle) changes related to aging (34). Given these potential changes in hormonal profile over time, it is unclear whether serum levels of hormones in the 5th and 6th decades of life correlate with hormonal status earlier in life (or throughout life), and whether a single hormone measure can represent one's hormonal status earlier in life.

Fourth, the effects of age at blood collection and age at diagnosis on the association between serum hormone levels and prostate cancer was rarely dealt with in previous nested case-control studies other than age-matching between cases and controls. In these prospective studies, age at blood collection can range from 45 to 65 years, age at diagnosis from 59 to 80 years, and duration of follow-up from 5 to 15 years. However, risks of prostate cancer in these nested case-control studies were estimated based on the simple categorization of subjects into tertiles or quartiles according to their serum hormone levels. For example, two subjects, one aged 45 years and the other aged 65 years at baseline, with similar serum levels of testosterone, would be classified into the same tertile (or quartile) regardless of their age at blood collection, age at diagnosis, length of follow-up, serum levels of

prostate-specific antigen, and aggressiveness of tumors (assessed by histologic grade). Given that serum testosterone levels decrease with advancing age, presumably serum levels of testosterone in the older subject (aged 65 years at blood collection) 20 years ago when he was 45 years of age would have been much higher than his serum testosterone levels at the baseline of follow-up. Nevertheless, according to most analyses in previous studies, he would be grouped in the same risk category with much younger subjects whose testosterone levels at the same age might have been very different. In theory, the levels of androgens at a particular age, the age of cancer diagnosis, and the aggressiveness of tumors are important biologic parameters in assessing the effect of androgens on prostate cancer. However, these factors are rarely taken into account in previous studies, perhaps due to small numbers and limited information on other covariates. If the effect of androgens is overwhelmingly large, a positive effect would prevail despite the "noise" or methodological limitations. However, in most studies, in both cases and controls, serum levels of testosterone are well within the normal range (500–1,200 ng/dl) and the case-control differences are quite modest (<15 percent) (3). Thus, more sophisticated approaches that incorporate both biologic and statistical perspectives are needed to examine the effect of serum androgens.

The most important limitation in epidemiologic studies assessing serum levels of androgens is whether circulating levels of androgens reflect androgenic activity within the prostate, since the metabolism of testosterone to dihydrotestosterone occurs mainly in the prostate, not in the circulation. If serum levels of androgens do not correlate with tissue dihydrotestosterone levels, it is difficult to interpret results from serologic studies.

Tissue-based studies

Despite the important concept that androgenic action in target tissue may be more relevant than serum levels of androgen in prostate carcinogenesis, no epidemiologic studies have assessed intraprostatic androgenicity directly, mainly due to difficulty in collecting prostate tissue from study subjects for the measurement of hormones. These collection problems are further compounded by the fact that it is not feasible to collect "normal" prostate tissue from healthy subjects for case-control comparisons.

Even if this obstacle to tissue collection could be overcome, reliably measuring hormones in tissue is another challenge. Because the texture, the amount of fibromuscular component, the proportion of epithelial cells, and the vascular patterns in each piece of tissue are likely to differ, their impact on androgen concentration, sample processing, the recovery of steroids during the extraction process, and reproducibility and accuracy of radioimmunoassays need to be taken into account.

It is even more difficult to assess enzymatic activity and the amount of androgen receptor proteins in prostate tissue. Although androgen receptor and its coactivators can be qualitatively and quantitatively measured through immunohistochemical staining, the accuracy and reliability of these measurements is still limited, partly due to the low specificity of antibody binding to these proteins (38, 39). No studies have yet measured these proteins along with tissue levels of hormones to derive an overall index of intraprostatic androgenicity that would elucidate further the role of androgens in prostate cancer etiology. In addition, because the 5α-reductase enzyme has not been purified, its activity cannot be measured directly. Thus far, the metabolic ratio of testosterone to dihydrotestosterone conversion in tissue is considered the best surrogate measure for this enzymatic activity. Methodological issues related to collection of prostate tissue in epidemiologic studies and variations related to tissue hormone assays have been summarized (A. W. Hsing, G. Hemstreet P. Levine, et al, unpublished data).

Current data in the literature on tissue hormones come mainly from clinical studies with very small numbers. Studies prior to 1990 mostly compared tissue hormone levels in patients with prostate cancer and benign prostatic hyperplasia, while those after 1990 focused mainly on the impact of finasteride, a competitive 5α-reductase inhibitor, on serum and tissue levels of androgens. The earlier studies were generally small, used less sensitive and specific assays to measure hormones in tissue, and failed to address several important methodological issues such as selection of subjects and comparability of tissue specimens between subjects. Therefore, these clinical data added little to our understanding of the role of tissue hormones in prostate cancer development.

Little is known about the correlations between serum and tissue hormones, factors affecting this relation, and intra- and inter-subject variability of tissue hormone assays. A better understanding of the hormonal milieu within the prostate and its relation with circulating hormones is critical to interpret results from serum-based studies and to expand our knowledge of the role of androgens in prostate cancer.

Non-androgenic hormones

Other than insulin-like growth factors, the role of non-androgenic hormones, including estrogens, insulin, leptin, vitamin D, and pituitary hormones, as well as sex hormone-binding globulin, is less understood. Data on insulin-like growth factors and vitamin D are summarized in other reviews in this special issue of *Epidemiologic Reviews*. This review will focus on estrogen, sex hormone-binding globulin, insulin, and leptin.

Estrogens and estrogen receptors. Estrogens in the prostate come from peripheral sources and aromatase activity within the prostate stroma (40). In addition, within the prostate the enzyme estrone sulfatase hydrolyzes estrone sulfate (E_1S) to estrone (E_1), which is then reduced to estradiol (E_2) by the enzyme 17β-hydroxysteroid dehydrogenase (encoded by *HSD17B*) (41, 42) (figure 2).

Although estrogen is used as an anti-androgen in the treatment of advanced prostate cancer, several lines of evidence suggest that estrogens may enhance the development of prostate cancer: 1) It has been shown that estrogens, mediated by sex hormone-binding globulin, participate with androgens in setting the pace of prostate growth and func-

tion (43). 2) Estrogens may also interact with the sex hormone-binding globulin receptor in the stroma to activate insulin-like growth factor synthesis and direct stromal proliferation, and through insulin-like growth factors, mediate the response of epithelial cells to androgens (42). 3) Experimental studies showing that induction of prostate tumors in laboratory rats by administration of testosterone is considerably enhanced with the addition of estradiol, suggesting that estrogens in conjunction with androgens may stimulate the development of prostate cancer (43). 4) Prenatal exposure to an extremely low dose of diethylstilbestrol and other estrogenic compounds produced a significant effect on mouse prostate development in vivo and in vitro in the presence and absence of androgen (42). Together, these laboratory data suggest that estrogens may enhance the risk of prostate cancer. It is possible that at a pharmacologic dose, estrogens may have anti-tumor action through their effect on the hypothalamic axis, while at physiologic levels, estrogens, as mitogens alone or in conjunction with androgens, may promote tumor growth. Five of the nine prospective studies evaluated the role of estradiol, and in the study of Gann et al. (25), after adjustment for sex hormone-binding globulin and androgens, serum levels of estradiol were inversely associated with prostate cancer risk. Current epidemiologic data on estrogens are too inconclusive to support a positive or inverse association.

The role of estrogen receptors is less clear and should be evaluated. There are two types of estrogen receptors, estrogen receptor-α and estrogen receptor-β. Although estrogen receptor-α is detected in stromal cells in the majority of molecular studies, it is presumably not highly expressed in prostate carcinoma, in contrast to estrogen receptor-β, which recent studies have found to be highly expressed in prostatic epithelial tissue (43–45). It has been suggested that estrogen receptors may affect prostate cancer risk through the influence of the estrogen-estrogen receptor complex on the concentration of androgen receptor (46). Based on the consistent synergistic effect of estrogens and androgens in inducing prostate tumors in laboratory studies, future epidemiologic studies should investigate the role of estrogen and estrogen receptor.

Sex hormone-binding globulin. Sex hormone-binding globulin is a glycoprotein produced and secreted by the liver and serves to transport androgens (testosterone and dihydrotestosterone) and estradiol. In addition to functioning as a carrier protein and a regulator of free fractions of testosterone and estrogens, it has been suggested that sex hormone-binding globulin has an effect on carcinogenesis independent of testosterone and estrogens (47). Sex hormone-binding globulin mediates steroid hormone signal transduction at the plasma membrane and plays a role in permitting certain steroid hormones to act without entering the cell through its interaction with the sex hormone-binding globulin membrane receptor (48, 49). It has also been shown that, through intermediacy of sex hormone-binding globulin, estradiol can activate the androgen receptor (50).

Because higher levels of sex hormone-binding globulin are associated with lower levels of free testosterone, sex hormone-binding globulin is thought to be associated inversely with prostate cancer risk. However, most studies have not evaluated the independent effect of sex hormone-binding globulin. Sex hormone-binding globulin levels are affected by several factors, including obesity, diabetes, and serum levels of testosterone, estrogen, insulin, leptin, insulin-like growth factors, and thyroid hormones (51–53). Future studies should evaluate the individual effects of sex hormone-binding globulin and its combined effects with testosterone and estradiol.

Insulin and leptin. Both insulin and leptin are involved in the regulation of body fat distribution and lipid and glucose metabolism (54, 55). Their roles in prostate cancer have been investigated in three previous studies (56–58). Of these, one nested case-control study in Sweden found a positive association with leptin but no significant association with insulin levels (56); the population-based case-control study in China reported a 2.5-fold risk of prostate cancer associated with higher levels of insulin, but the positive association with leptin disappeared after adjustment for insulin and insulin-like growth factors (57); and a small case-control study in Greece found no association with leptin (58) (table 1). Each of these studies has its own limitations. The nested case-control study in Sweden included non-fasting or 4-hour fasting samples, the study in China is cross-sectional and cannot completely rule out the potential impact of a disease effect on the measurement of insulin and its association with prostate cancer, while the Greek study is very small (43 cases and 48 healthy controls).

The association with insulin and leptin is intriguing and the hypotheses are biologically plausible, although they are too preliminary to suggest a role for insulin or leptin in prostate cancer etiology. Insulin may affect prostate cancer through the obesity-hormone or insulin-like growth factor pathways. Figure 5 shows how insulin may influence the risk of prostate cancer biologically. Insulin can affect androgen biosynthesis and metabolism through *CYP17*, *CYP19* (59–62), and circulating levels of sex hormone-binding globulin (63, 64). The insulin system stimulates P450c17 mRNA expression and activities in the adrenal glands. In addition, insulin can affect the uptake of androgen receptor in vivo (65), thus influencing androgenic action in the prostate gland. In cross-sectional studies, serum levels of insulin correlated negatively with testosterone but positively with 3α-diol G (57, 66), further suggesting that insulin may influence prostate cancer risk through changes in the hormonal milieu within the prostate gland.

In addition to the androgen pathway, insulin may affect prostate cancer risk through the insulin-like growth factor system. Insulin-like growth factor-I has been implicated in the regulation of prostate epithelial cell proliferation and in the etiology of prostate cancer (67–69). Like insulin-like growth factor-I, insulin is a mitogen, appears to be a growth factor for prostatic epithelial cells, and has an anti-apoptotic effect (70). Insulin down-regulates insulin-like growth factor binding protein-I, thereby increasing the bioavailability of insulin-like growth factor-I (71). The receptors for insulin and insulin-like growth factor-I are homologous; thus, insulin can bind to and activate the insulin-like growth factor-I receptor (67, 72).

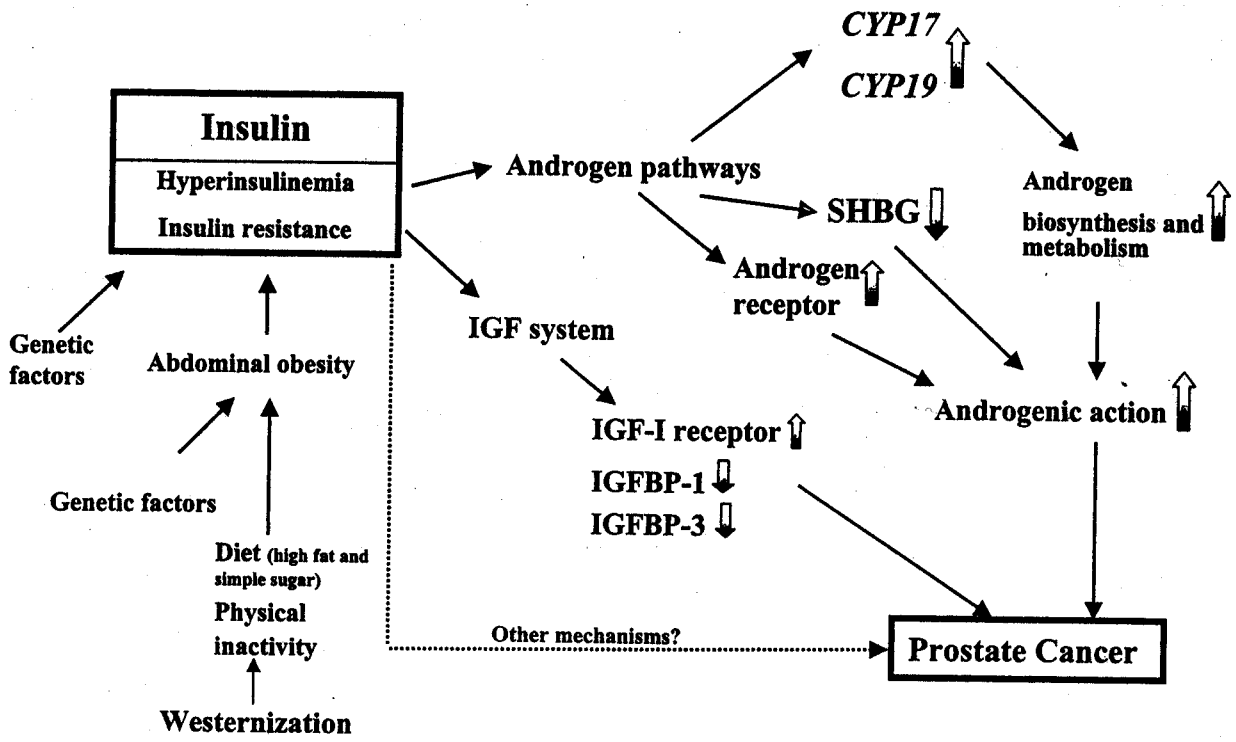

FIGURE 5. Hypothetical relation between insulin and prostate cancer. Abbreviations: IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; SHBG, sex hormone-binding globulin.

Few studies have investigated the role of insulin in prostate cancer, partly due to circulating levels of insulin being affected by recent food intake, the relatively short half-life of insulin, and the need to use overnight fasting samples for the measurement of insulin.

Leptin is the protein of the obesity (*Ob*) gene and is related to fat distribution and sex hormones. Leptin increases the amount of adipose tissue in the body (73, 74), regulates food intake and energy balance (75, 76), and interacts with other endocrine systems (77, 78), whereas insulin increases leptin gene expression, stimulates leptin protein production in rats, and regulates leptin and sex hormone-binding globulin protein levels in vivo and in vitro (79).

Studies of migrants in the United States have shown that westernization is associated with an increased risk of prostate cancer (80). Given that obesity and insulin resistance may be important consequences of westernization, insulin/leptin may be a fruitful area for future research.

FUTURE DIRECTIONS

Prostate cancer is a heterogenous disease; thus, a complete picture of prostate cancer etiology probably involves intricate biologic interplays among hormones, hormone-metabolizing genes, receptor proteins, and exogenous factors. In order to elucidate further the role of hormones, we need to tackle this problem from several different angles with more creative approaches. These include an array of rigorously conducted methodological studies to shed light on serum-tissue correlations, determinants of hormones, and racial differences in hormone levels. Insights gained from these methodological studies can then be applied to future prospective studies to refine exposure assessment, classification of outcome, and data analysis. Table 3 and figure 6 outline the various approaches needed to tackle these problems.

METHODOLOGICAL STUDIES

Studies of prostate tissue

The key question that needs to be answered is whether circulating levels of hormones reflect androgenic action within the prostate. It is important to differentiate androgen levels from androgenic action. Well-designed rigorously conducted methodological studies should be carried out to collect high quality fresh snap-frozen normal prostate tissue for the measurement of tissue hormones, enzymatic activities, and receptor proteins so that an overall index of androgenicity in the prostate can be derived. Such studies are logistically challenging; thus, meticulous attention should be paid to details related to setting up the infrastructure for subject selection, recruitment, tissue procurement, collection procedures, and validation of hormone assays. Quality control procedures should be implemented to evaluate intra-

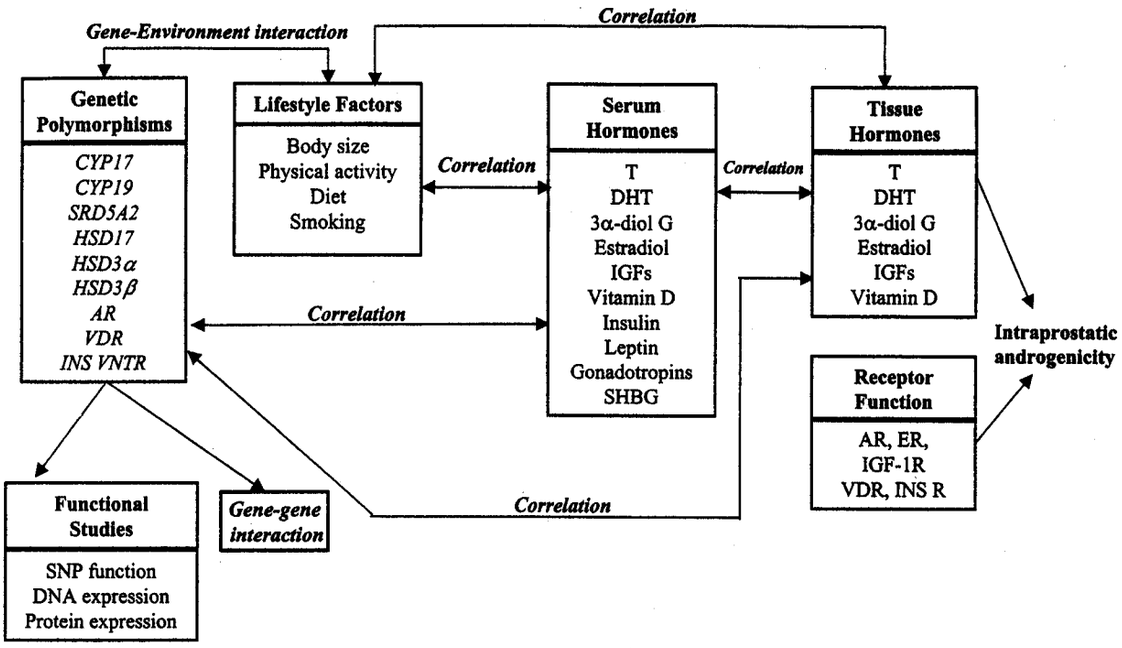

FIGURE 6. Future research. Abbreviations: AR, androgen receptor; DHT, dihydrotestosterone; 3α-diol G, 3α-androstanediol glucuronide; ER, estrogen receptor; IGFs, insulin-like growth factors; IGF-1R, insulin-like growth factor-1 receptor; INS R, insulin receptor; SHBG, sex hormone-binding globulin; SNP, single nucleotide polymorphisms; T, testosterone; VDR, vitamin D receptor.

prostatic as well as intra- and inter-assay variation in tissue hormones. Once tissue hormone assays have been validated, it is essential to assess whether smoking, alcohol use, body size, and other lifestyle factors affect tissue levels of hormones. Racial/ethnic differences in tissue levels of hormones should be evaluated after taking lifestyle and other potential confounding factors into account. Some work has been initiated in this area and the details necessary for such a study have been compiled (A. W. Hsing, G. Hemstreet, P. Levine, et al., unpublished data).

In these methodological studies, on the same day that tissue is collected from prostatectomy, fasting blood samples should be collected prior to surgery so that circulating levels of hormones can be measured and compared with tissue levels. If possible, such studies should be conducted in various racial/ethnic groups in order to understand whether racial/ethnic differences exist in serum-tissue correlations. The correlation between serum and tissue levels of hormones partly reflects hormone metabolism within the prostate and thus may provide insights into whether intraprostatic metabolism is more etiologically relevant than serologic measurement. If large enough tissue samples can be collected (>400 mg), metabolism studies need to be carried out to estimate the conversion ratio of testosterone to dihydrotestosterone in tissue. This ratio is thus far the best possible measure of the steroid 5α-reductase 2 activity in the prostate, since this enzyme has not been purified and currently it is not possible to measure the enzymatic activity directly. Racial/ethnic differences in the intraprostatic testosterone/dihydrotestosterone conversion ratio would provide important support for the hypothesis that differences in the enzymatic activity of 5α-reductase within the prostate gland can explain most of the racial/ethnic differences in prostate cancer risk (81, 82).

In these methodological studies, peripheral lymphocytes or buccal cells should also be collected in order to purify genomic DNA for the assessment of genetic susceptibility related to hormone metabolism. Correlations between tissue and serum levels of hormone (phenotypes) and polymorphisms (genotypes) of hormone-metabolizing genes, including *CYP17*, *CYP19*, *SRD5A2*, *HSD3B*, and *HSD17B*, should be evaluated to provide insights into the functional significance of these genetic markers (3, 81).

Population-based cross-sectional studies

Although tissue-based studies are important, in epidemiologic studies it is not possible to compare tissue levels of hormones in cases and controls or, in prospective studies, to measure tissue levels of hormones at baseline. Obviously, future studies will continue to rely on serum-based measurements. Thus, it is important to derive solid data from methodological studies to aid in the interpretation of future nested case-control studies.

Multi-ethnic comparisons. Because race/ethnicity remains one of the best clues to prostate cancer etiology, a better understanding of when and how serum levels of certain hormones vary in different populations may shed light on the

etiology of prostate cancer. It has been suggested that differences in hormone levels in various racial/ethnic groups may account for part of the differences in prostate cancer risk. Nevertheless, very few studies have examined population differences in hormone levels in various racial/ethnic groups. We need to conduct population-based cross-sectional studies in several racial/ethnic groups to determine the following: 1) whether there are racial differences in circulating levels of hormones; 2) if differences exist and in which decade of life the racial/ethnic differences in hormone levels are most apparent (this information will help identify the etiologically relevant time periods in hormone carcinogenesis and prostate cancer); 3) the impact of lifestyle factors on hormone levels and an assessment of whether either race or ethnicity modifies these differences; 4) the allele frequency of polymorphisms of hormone-related genes in various racial/ethnic groups (this will help us to assess the role of genetic factors in racial/ethnic differences in prostate cancer risk); and 5) to correlate genotypes (genetic susceptibility) with phenotypes (serum or tissue levels of hormones) in these groups to understand whether race/ethnicity and lifestyle factors modulate the expression and function of these genetic traits.

The suggested methodological studies, although cross-sectional in nature, should be guided by sound epidemiologic principles in order to provide solid data to aid in the interpretation of results from future prospective studies. For example, in order to derive valid estimates in each population for racial comparisons, these studies should be conducted with a common standardized protocol, using probability samples selected from each population. The study should be large enough in each racial/ethnic group (preferably several hundred subjects) to provide sufficient power to evaluate combined effects of several hormones and meaningful population differences. These studies should also include men from various age groups, in particular those in the younger age groups (<30 years of age), since earlier reports suggested that the differences in serum levels of testosterone between African-Americans and Caucasians were most pronounced in younger men but that such racial differences disappeared gradually with advancing age (83). In these future studies, information on epidemiologic characteristics, such as body size, physical activity, diet, diabetes, and other hormone-related factors, should be collected so that the impact of lifestyle on serum hormones in various populations can be compared. If several racial/ethnic groups can be successfully assembled for such a cross-sectional comparison, and if resources are available, it is recommended that this group of subjects (several hundred in each group) be followed over time with multiple collections of biologic samples from individuals so that changes in hormone levels over time in relation to lifestyle factors in various racial/ethnic groups can be evaluated.

Studies like this rarely include very young children. However, it has been suggested that early life events and in utero exposure to androgens may be important in the development of prostate cancer, and that racial/ethnic differences in androgen levels in pregnant women may contribute partly to the large racial/ethnic difference in risk (37). Although it is logistically difficult, this hypothesis is worth exploring. Cord blood samples from women of a similar gestational age in high- and low-risk populations should be collected to assess racial/ethnic differences in intra-uterine exposure to androgens.

Surrogate hormone markers. Endogenous hormones remain one of the most important hypotheses in prostate cancer etiology. It is obvious that measuring hormone levels in human subjects at etiologically relevant time points is not a trivial task. Hormone levels vary with age, lifestyle changes, and perhaps other as yet unidentified factors, making it difficult to develop an index to characterize a specific or cumulative exposure over time for a particular subject. Unlike women, men do not have clear life events, such as menarche, first birth, lactation, and menopause, that mark the key hormonal changes in their lives, although it is well documented that andropause occurs in men and that androgen levels in men decrease with advancing age. Despite the difficulty, it is necessary to expend the effort to develop some kind of hormone indices for men for use in large-scale epidemiologic studies to characterize their hormonal status over time. For example, there are clearly racial/ethnic differences in male-pattern baldness, chest hair density, body odor, sweating patterns, ear wax dimorphism, fertility, body size, age at onset of puberty, and physical development during puberty (84–90). All of these phenotypes have an androgen component. For example, male pattern baldness and chest hair density are associated with dihydrotestosterone metabolism in the skin and polymorphisms of androgen receptor (90, 91). Although these hormone-related questions are quite varied, the extent of misclassification may be no greater than that from a dietary interview. Methodological studies should be conducted to refine further these key questions and validate them by biomarkers before they can be applied to epidemiologic studies as surrogate measures to reflect the hormonal status of men over time.

Genetic markers. With the availability of molecular tools, there has recently been a shift from serum-based studies to the investigation of genes involved in hormone biosynthesis, metabolism, and transport. Most molecular epidemiologic studies currently investigate common polymorphisms without much information regarding functional consequences. This approach may lead to difficulties in the interpretation of results because there are numerous polymorphic markers. For example, a particular association may be mechanistically significant or merely reflective of linkage to another truly causative marker, since there is little functional data to suggest the molecular significance of certain single nucleotide polymorphisms. These kinds of uncertainties may explain at least some of the often contradictory outcomes reported in various molecular epidemiologic studies (3). Because genes act in concert, it is likely that a set of genetic polymorphisms, rather than a single polymorphism, can alter hormone levels and prostate cancer risk. Whenever possible, serum/tissue levels of hormones should be measured in conjunction with genetic polymorphisms. Correlations between these markers provide some insights into the functional significance of any genetic variant under investigation. Polymorphisms and hormone levels should be considered to be sequential,

rather than interacting, variables. For example, a polymorphism in a synthesis gene could affect prostate cancer risk through altering hormone levels as an intermediate variable. Whether the frequencies of these genes within and between different racial groups are related to prostate cancer risk and contribute to the large differences in risk needs further confirmation through population-based studies integrating epidemiologic and molecular components.

PROSPECTIVE STUDIES

Because it is not feasible to compare tissue hormone levels in case patients and healthy men in epidemiologic investigations, future studies will continue to depend on serum-based studies. Obviously, prospective studies in various populations will be the gold standard to provide data regarding the role of hormones in prostate cancer. However, the conventional approach used in previous studies, which included small numbers of cases, measured a limited number of hormones, did not consider confounding, or did not take into account the potential impact of age at blood collection, age at diagnosis, or the aggressiveness of tumors, is not likely to shed new light on the hormonal hypothesis. To break new ground, nested case-control studies in the new millennium need to include a large enough sample size (more than several hundred cases) to permit the measurement of several hormones at the same

TABLE 3. Suggested future directions for studies on hormones and prostate cancer

Methodological studies

I. Serum-tissue correlations
- Examine correlation between serum and tissue levels of hormones (preferably in non-diseased prostates)
- Evaluate correlation between intraprostatic androgenicity (tissue hormones, growth factors, androgen receptor and coactivators of androgen receptor levels) and serum levels of hormones in order to determine which serum markers best reflect intraprostatic androgenicity
- Assess genetic polymorphisms and their relations with serum or tissue levels of hormones to gain insights into the functional significance of these genetic markers
- Assess the impact of lifestyle factors on tissue levels of hormones

II. Population-based cross-sectional studies
- Examine serum hormone levels in various racial/ethnic groups in population-based probability samples in different age groups to identify in which age groups serum levels of hormones differ, if any, in these racial/ethnic groups to understand better the etiologically relevant time periods in prostate carcinogenesis
- Evaluate determinants of serum androgens, including the impact of lifestyle factors, in order to develop better analytical models to assess the independent effect of a particular hormone
- Evaluate whether factors affecting hormone levels vary by race/ethnicity
- Measure several hormones, including androgens, estrogens, sex hormone-binding globulin, insulin, leptin, and growth factors, simultaneously in order to evaluate the interrelation among these serum factors. This information is useful for adjustment for confounding in future analyses and may provide a more complete hormone profile for each subject
- Use results from tissue-serum correlation studies to adjust for risk estimates, if necessary
- Include large samples (preferably several hundred subjects in each racial group) to provide enough numbers for subgroup analysis
- Adjust for other confounders (collect information on other epidemiologic risk factors)
- Evaluate the role of hormones in subjects by different types of genetic susceptibility

III. Other methods studies
- Compare serum levels of hormones in incident cases with those of controls in prospective studies, if possible, in various racial/ethnic groups

Prospective studies

I. Exposure assessment
- Use nested case-control studies within the cohort to evaluate the role of hormones in various racial/ethnic groups
- Include large sample size (at least several hundred cases)
- Measure epidemiologic risk factors in order to control for confounding
- Measure several hormones involved in a specific metabolic pathway simultaneously
- Use assays with high sensitivity, specificity, and reliability such as liquid chromotography/mass spectrometry
- Use results from tissue-serum correlation studies to adjust risk estimates, if necessary

II. Outcomes
- Collect detailed information on case characteristics, including clinical stage, histologic grade, and serum levels of prostate-specific antigen

III. Statistical issues
- Incorporation time factors (age at diagnosis and age at blood collection) into data analysis
- Control for confounding from other hormones or hormone-related risk factors, if present
- Evaluate the effect of hormones in subgroups (e.g., obese vs. non-obese) separately
- Evaluate the effect of hormones by genetic susceptibility separately
- Evaluate the effect of hormones by stage and grade of tumors separately
- Evaluate the possibility of gene-hormone or hormone-environment interactions

time, thus providing a more complete hormone profile and permitting the evaluation of potential individual and combined effects of several hormones simultaneously (table 3). For example, recent studies suggest that the insulin-like growth factor axis may cross-talk with the vitamin D receptor pathway and that insulin may affect prostate cancer risk through either the hormonal or the insulin-like growth factor pathways (92); some of these may also affect the signaling transduction of androgen receptor within the prostate gland. Genetic susceptibility should also be assessed in these studies, since it is possible that the effect of hormones is not the same across populations and that a subset of a population is more susceptible to hormone exposure. For example, the same concentration of testosterone may have substantially different effects on individuals, given the differences in the CAG repeat length in their *AR* gene and the genetic susceptibility of other hormone-related genes. Thus, a more integrated approach is needed to sort out the individual and combined effects of these hormones, gene-gene interactions, and gene-environment interactions. Information on epidemiologic factors, in particular those related to hormone metabolism, such as body size, physical activity, and diabetes, should be collected to permit adjustment for confounding by these factors. Information on clinical parameters, such as clinical stage, histologic grade, and serum levels of prostate-specific antigen, should be collected as well in order to analyze hormone data based on more accurate classification of disease, since the effect of testosterone on aggressive and relatively indolent tumors is likely to differ. If possible, future studies should employ state-of-the-art assays, such as liquid chromatography/mass spectrometry (93), to minimize measurement error and intra- and inter-assay variation and provide a much more precise exposure assessment.

SUMMARY

In summary, the hormonal hypothesis remains one of the most important hypotheses in prostate cancer etiology. Although epidemiologic data regarding the role of hormones are still inconclusive, there are many intriguing leads. Armed with more complete methodological data, state-of-the-art hormone assays, sound epidemiologic design, and a more thorough analytical approach, a new generation of studies should yield critical data and insights to help clarify further the role of hormones in prostate cancer. These new studies may determine ultimately whether racial/ethnic differences in hormonal levels and in genetic susceptibility to hormone-metabolizing genes can help explain the very large racial/ethnic differences in prostate cancer risk.

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